RECOMBINANT LUBRICINS, AND COMPOSITIONS AND METHODS FOR USING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent application No. 62/792,660, filed Jan. 15, 2019, the entire disclosure of which is incorporated herein by reference.

GOVERNMENT FUNDING

[0002] This invention was made with government support under grant nos. 1DP2GM119133-01 and 1U54CA210184-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

[0003] The disclosure provided improved glycoproteins, and compositions and methods related to the same.

BACKGROUND OF THE DISCLOSURE

[0004] Lubricin is a glycosylated protein found in several places in mammalian anatomy. For example, lubricin is present in synovial fluid and on the surface of cartilage. Lubricin has an important role in lubrication of joints and maintaining the correct joint environment.

[0005] Previous attempts have been made to provide recombinant forms of lubricin, but there remains an ongoing and unmet need for new lubricin and lubricin-like glycoproteins that can be employed in a wide variety of environments. The present disclosure is pertinent to this need.

SUMMARY OF THE DISCLOSURE

[0006] The present disclosure provides compositions and methods that relate to modified glycoproteins. Aspects of the disclosure pertains to modified lubricins, pharmaceutical compositions that contain the modified lubricins, cDNAs and expression vectors that encode the modified lubricins, eukaryotic cells that express the modified lubricins, and methods of using the modified lubricins and compositions comprising them for a variety of purposes. The methods include use of such agents for prophylaxis and/or therapy of a variety of conditions where improved lubrication of a surface or fluid within a human or a non-human mammal is desirable. The disclosure also includes using the compositions to provide lubrication to the surface of a variety of inanimate objects.

[0007] In certain embodiments, the modified lubricins comprise a change in a number of tandem repeats of specific amino acid sequences, and/or one or more changes in the amino acid sequences of the modified lubricins, relative to their naturally produced counterparts. In embodiments, the modified lubricins comprise amino acid sequences that are derived from human, equine, or canine lubricins, but have different functional attributes relative to previously provided recombinant versions of such sequences. In an embodiment, the modified lubricins have an increased half-life, such as an intra-articular half-life when injected into a mammal, of more than 4 days. In embodiments, the modified lubricins exhibit an intra-articular half-life of more than 15 days, or at least 30 days. In embodiments, the modified lubricins have a modified glycosylation pattern, relative to an unmodified lubricin.

[0008] In embodiments, the modified lubricins include contiguous repeated sequences that are one or a combination of KEPAPTTP (SEQ ID NO:1), KEPAPTP (SEQ ID NO:9) and KEPAPTTTP (SEQ ID NO:10). In embodiments, the repeated sequence is repeated contiguously 10-120 times. In a non-limiting embodiment, the repeated sequence is repeated 59 times.

[0009] In embodiments, the modified lubricins comprise amino acid sequences that are derivatives of lubricins produced by human or non-human mammals. In embodiments, the contiguous repeated sequences are flanked on their N- and C-terminal segments by lubricin amino acid sequences that are at least 90% identical to human, equine, or canine lubricin sequences.

[0010] In embodiments, the modified lubricins include additional components, such as an added secretory signal from a human, or a non-human mammal, or other suitable source.

BRIEF DESCRIPTION OF THE FIGURES

[0011] The figures and tables of this disclosure are divided into four Parts (Part I, Part II, Part III and Part IV), as described below.

Part I Figures

[0012] FIG. **1**: Combinatorial Genetic Encoded Library for Sequence-Specific Mucins. (a) Schematic diagram of the combinatorial sequence-specific mucins. (b) Schematic shows the swappable bio-bricks and flanking restriction sites for complete mucin construction. (c) Work flow for the design and fabrication of cDNAs for the mucin tandem-repeat backbones. (d) Summary of codon-scrambled mucin backbones in the library. The Wild-type Muc1 sequence is SEQ ID NO:8. The Muc1 single mutant (Muc1_S) is SEQ ID NO:5. The Muc1 double mutant (Muc1_D) is SEQ ID NO:6. The Muc1 triple mutant (Muc1_T) is SEQ ID NO:7. The Synthetic 1 (Syn1) is DAATPAP is SEQ ID NO:2. The Synthetic 2 (Syn2) is SEQ ID NO:3. The Synthetic 3 (Syn3) is SEQ ID NO:4. The Lubricin consensus sequence (Syn4) is SEQ ID NO:1.

[0013] FIG. **2**: Construction and Validation of Sequence-Specific Mucin Expression. (a) Components and features of codon-optimized Muc1 variants with GFP reporters. The amino acid sequence in (a) is SEQ ID NO:8 (b) Predicted Molecular Weight of the polypeptide backbone. (c) Biosynthesis of Tn antigen, Core 1, and Core 2 glycans, and specificity of relevant lectins for their detection. (d) Western Blot analysis of Native Muc1 expression and glycosylation in wild-type and Core-1 β3-T specific molecular chaperone (COSMC) knockout MCF10A cells. The MCF10A cells were stably transfected with native Muc1. The surface sialic acids were labeled with AFDye 568 through periodate labeling prior to lysate collection. The blot was stained in multiple colors with MUC1 TR (CD227 HPMV) Ab-FITC, and PNA-CF640 or biotinylated VVA (Secondary: NeutrAvidin-Dylight 650). (e) Western blot analysis of native and codon-scrambled Muc1 in extracts of transiently trnsfected HEK293T cells. (f) Immunofluorescence images of transiently transfected HEK293T cells expressing indicated constructs and probed with PNA lectin (left), anti-Muc1 anti-